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SERUM PROTEIN-BOUND CARBOHYDRATES AND OTHER GLYCOPROTEIN ASSAYS--ETC(U)
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# SERUM PROTEIN-BOUND CARBOHYDRATES AND OTHER GLYCOPROTEIN ASSAYS AS INDICATORS OF TUMOR BURDEN,

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Elevations in serum glycoproteins and protein-bound carbohydrates (PBC) are significant consequences of radiation damage, trauma, and certain disease states such as cancer. Thus, it is important to determine the diagnostic, prognostic, and functional significance of the glycoprotein elevations in various injuries and diseases. Levels of glycoprotein-associated carbohydrates (neutral hexoses, hexosamines, sialic acid, and fucose) were determined in the serum of patients with either local,

20. ABSTRACT (continued)

regional or metastatic cancer, patients clinically cured of cancer, and controls (smokers and nonsmokers). Total protein-bound carbohydrates were compared to levels of 17 normal serum glycoproteins, carcinoembryonic antigen (CEA), and to lymphocyte reactivity to phytohemagglutinin (PHA). Tumor burden was directly related to protein-bound carbohydrate levels in patient groups. Levels of bound carbohydrates reflect the sum of all the changes in serum glycoproteins, but primarily changes in the acute-phase proteins ($\alpha_1$-acid glycoprotein, $\alpha_1$-antitrypsin, haptoglobin, and ceruloplasmin) found in the $\alpha$-globulin fraction of serum. Serum protein-bound carbohydrates, such as sialic acid, appear to be better tumor markers than CEA in patients with various types of solid malignancies. Increases in sialic acid in tumor-bearers do not appear to correlate with increases in CEA, suggesting that a "nonspecific" tumor marker and a "tumor-derived" marker may complement each other in assessing patient status. Increased levels of the acute-phase proteins and decreased levels of $\alpha_2$HS-glycoprotein occur in individuals with depressed in vitro lymphocyte reactivity to PHA, but the relationship between lymphocyte reactivity and specific serum glycoproteins needs further investigation. The present study increases our understanding of the relationship of serum glycoproteins to disease and injury.

alpha

## PREFACE

The carcinoembryonic antigen (CEA) assay was performed by J. P. Vandevoorde and co-workers at the Cancer Diagnostic Laboratory, Hoffmann-La-Roche Inc., Nutley, New Jersey. We thank M. J. Ryan, M. L. Nelson, W. W. Wolfe and K. M. Hartley for their assistance and A. S. Evans for his advice and encouragement.

## TABLE OF CONTENTS

## LIST OF FIGURES



## LIST OF TABLES

## INTRODUCTION

It is known that the majority of serum proteins contain bound carbohydrates but the function of these glycoproteins is extremely varied, and in some cases is unknown.[4,21,34,39] Variations in serum glycoprotein levels are characteristic of many pathological conditions, particularly inflammation, infection, trauma, and malignancy.[7,23,36,38] In the past, there have been many approaches to the investigation of glycoproteins in cancer.[3,46] Changes in concentration or composition of normal serum glycoproteins or electrophoretic fractions, such as $\alpha$-globulins and immunoglobulins, have been investigated extensively.[1,2,31,34,38,48] Seromucoid, a carbohydrate-rich fraction of serum proteins, has been studied as an indicator of tumor presence.[17,45] Differences in glycoproteins can be measured by determining their carbohydrate moieties.[23,45] For example, increases in the protein-bound carbohydrate, fucose, have been shown in patients with breast cancer,[27,29] solid gynecologic malignancies,[5] and cancer patients in general.[14,32] There are serum glycoproteins in very low concentrations that increase during malignancy, including hormone-related substances, such as human chorionic gonadotrophin (HCG),[40] and enzymes, such as ribonuclease.[10] Over and above an increase in normal serum glycoproteins, neoplasia may be accompanied by elevations of new or "tumor-specific" molecules, such as carcinoembryonic antigen (CEA) and $\alpha$-fetoprotein.[22]

In addition to studies on changes in serum glycoproteins in neoplasia, a separate line of investigation has focused on the functional influence of glycoproteins on humoral and cell-mediated immunity. Depression of cellular immunity and serum-mediated immune modulation during malignancy have been demonstrated in our own and other laboratories.[9,33,41] Identification of the factor(s) in the sera of cancer patients that suppress lymphocyte reactivity appears pertinent to the understanding of the alteration of the host-cancer relationship. Immunosuppressing activity in human serum from cancer patients has been related to increases in the $\alpha$-globulin fraction[12] or sialic acid levels.[43]

Other research has demonstrated further the immunological importance of $\alpha$-globulins. Increased tolerance to parabiosis and an increased take of skin grafts in animals, caused by exogenously administered $\alpha$-globulin, have been reported.[19]

The purpose of the present study was to describe the changes in levels of glycoprotein-associated monosaccharides (neutral hexoses, fucose, hexosamines, sialic acid), and their relationship to tumor burden. The protein-bound carbohydrates were compared to changes in the serum concentrations of 17 normal glycoproteins synthesized by the liver or lymphoid tissue, and to serum CEA levels. Lymphocyte reactivity to phytohemagglutinin (PHA) was determined for some of the individuals in an attempt to determine how these glycoprotein measurements compared to an immunological parameter as indicators of tumor burden.

## MATERIALS AND METHODS

Groups studied. The patient population was seen, treated, and followed by the Surgery Branch of the National Cancer Institute. Serum levels of glycoprotein-associated carbohydrates were determined in 150 individuals. Of 105 cancer patients tested, 62 were tumor-bearing with no previous therapy, and 43 were clinically cured. The 62 tumor-bearing individuals included patients with the following diagnoses: 29 squamous cell carcinomas (23 head and neck primaries and six cervical primaries); 10 melanomas; 10 sarcomas; 8 adenocarcinomas of the breast; and 5 adenocarcinomas of the colon.

Forty-three individuals, clinically cured for at least 2 years, were chosen to match histologically those in the tumor-bearing group. They had been cured of the following tumors: 12 head and neck and 2 cervical squamous carcinomas; 10 melanomas; 9 sarcomas; 5 adenocarcinomas of the breast; and 5 adenocarcinomas of the colon. Cured and tumor-bearing cancer patients were classified for analysis according to tumor burden as either (having or having had) local, regional, or systemic disease.

A nonsmoking control group of normal individuals was studied and consisted of 13 females and 17 males whose sex and age distribution paralleled

that of the cancer group. An additional control group of 15 age- and sex-matched volunteers who were heavy cigarette smokers was also tested. The mean cigarette consumption in this group was equivalent to 30 pack-years (1 pack-year = 1 pack/day/year).

Serum CEA levels were determined in 130 patients and controls. This group consisted of 27 healthy normals, 15 heavy smokers, 53 tumor-bearers, and 35 clinical cures. Levels of specific normal serum proteins were determined in 10 controls, 43 tumor-bearers, and 39 clinical cures. Lymphocyte reactivity was determined in 27 healthy normals, 15 heavy smokers, 35 tumor-bearers, and 23 clinical cures.

Carbohydrate determinations. Blood samples were obtained by sterile venipuncture and allowed to clot at 37°C. Serum was separated by centrifugation and stored frozen. Samples from the entire test group were analyzed simultaneously to minimize variability in the assay systems. Total protein levels were determined by the biuret method. Globulin levels were determined by a copper sulfate-tryptophan color reaction using commercially available reagents (Hycel, Inc., Houston, Texas). The separate protein-bound carbohydrate (PBC) levels were determined using color reactions specific for each sugar.[45] Hexosamine, neutral hexose, and fucose levels were determined in an ethanolic precipitate of serum. Hexosamine levels were determined using a modification of the Elson-Morgan reaction. The neutral hexose levels were determined using the orcinol reaction, and the fucose levels were determined using a modification of the Dische-Shettles reaction.[14] Sialic acid levels were determined by the diphenylamine reaction using a trichloroacetic acid precipitate of serum.

Specific serum proteins. Eighteen specific serum proteins were determined by radial immunodiffusion using commercially available (Behring Diagnostics, Somerville, New Jersey) plates and control serums. The specific proteins assayed were: albumin, $\alpha_1$-acid glycoprotein, $\alpha_1$-antitrypsin, ceruloplasmin, haptoglobin, Gc-globulin, $\alpha_2$-macroglobulin, $\alpha_2$HS-glycoprotein,

$\beta$-lipoprotein, transferrin, hemopexin, $\beta_2$-glycoprotein, $\beta_1$A-globulin, $\beta_1$E-globulin, IgM, IgA, IgG, and plasminogen. CEA levels were determined in serum using the Hansen Z gel assay.[16]

Lymphocyte reactivity. Lymphocyte reactivity to PHA was determined using Ficoll-Hypaque separated lymphocytes in a microculture technique. The technique has been described previously,[37] and briefly consists of a 72-hour culture of 150,000 mononuclear cells at $37^{\circ}C$ exposed to varying doses of PHA. Tritiated thymidine was added 6 hours prior to termination of the culture as a pulse, and reactivity was determined by harvesting the lymphocytes in a Mash unit and counting incorporation of $H_3T$ in a scintillation counter. Each culture was done in triplicate and the reported result for each determination is the mean of the peak triplicate sample minus mean background activity.

Statistics. The significance of differences between means was evaluated using Student's "t" test. Correlation coefficients were determined between biochemical and immunological parameters. Differences or correlations were considered significant if $p < .05$.

## RESULTS

The mean levels of total protein, globulin, and the protein-bound carbohydrates (neutral hexose, hexosamine, sialic acid, and fucose) were calculated for each of the various patient and control groups (Table 1). When the mean protein-bound carbohydrate levels of all tumor-bearing individuals were compared to the levels found in the normal test population, both smokers and nonsmokers, statistically significant elevations were found in the levels of each. When tumor-bearing patients were separated according to tumor histology, in general, patients with squamous tumors had a higher elevation of protein-bound carbohydrates (Table 2). The elevations found in either of the tumor-bearing groups, squamous or nonsquamous, were significantly different from normal (Table 2) and, for the comparison of protein-bound carbohydrate results with other data, will be considered together.

Table 1. Serum Protein-Bound Carbohydrates in Patients With Solid Tumors

| | Total protein (mg/ml) | Globulin (mg/ml) | Neutral hexose (mg/100 ml) | Hexosamine (mg/100 ml) | Sialic acid (mg/100 ml) | Fucose (mg/100 ml) |
|---|---|---|---|---|---|---|
| Healthy normals (30) | 66.3 ± 4.4 | 28.1 ± 3.7 | 121.0 ± 12.3 | 78.6 ± 9.0 | 69.7 ± 9.2 | 4.2 ± 0.9 |
| Heavy smokers (15) | 66.4 ± 3.6 | 27.5 ± 2.4 | 126.0 ± 11.6 | 87.4 ± 8.6† | 72.1 ± 8.6 | 5.0 ± 1.2* |
| Tumor-bearing | | | | | | |
| Local (23) | 65.0 ± 5.9 | 29.2 ± 3.7 | 138.0 ± 17.7‡ | 86.9 ± 12.7† | 86.2 ± 16.1‡ | 5.6 ± 1.6‡ |
| Regional (28) | 66.1 ± 6.4 | 31.6 ± 5.2* | 160.6 ± 33.9‡ | 100.8 ± 23.9‡ | 100.2 ± 24.5‡ | 6.8 ± 3.0‡ |
| Systemic (11) | 62.5 ± 3.7* | 30.9 ± 5.8 | 179.2 ± 60.1‡ | 111.2 ± 40.8‡ | 119.0 ± 48.3‡ | 7.6 ± 4.8‡ |
| All tumor-bearers (62) | 65.1 ± 5.9 | 30.6 ± 4.9* | 155.5 ± 38.0‡ | 97.5 ± 25.8‡ | 98.4 ± 29.6‡ | 6.5 ± 3.0‡ |
| Cured clinically (43) | 69.5 ± 6.3* | 29.5 ± 4.0 | 135.5 ± 18.1‡ | 83.3 ± 11.7 | 80.4 ± 16.1† | 5.1 ± 1.8* |

Values are means ± standard deviations
Significance of difference compared to healthy normals
* $p < .05$
† $p < .01$
‡ $p < .001$

Table 2. Comparison of Serum Protein-Bound Carbohydrates in Patients With Squamous and Nonsquamous Tumors

| | Total protein (mg/ml) | Globulin (mg/ml) | Neutral hexose (mg/100 ml) | Hexosamine (mg/100 ml) | Sialic acid (mg/100 ml) | Fucose (mg/100 ml) |
|---|---|---|---|---|---|---|
| Tumor-bearing | | | | | | |
| Squamous, local (10) | 67.6 ± 5.2 | 30.5 ± 4.0 | 140.6 ± 17.4* | 88.0 ± 13.5* | 81.7 ± 13.8* | 6.5 ± 1.5*† |
| Nonsquamous, local (13) | 63.0 ± 5.8 | 28.1 ± 3.1 | 136.0 ± 18.4* | 86.0 ± 12.6* | 89.7 ± 17.3* | 4.9 ± 1.4*† |
| Squamous, regional (17) | 66.3 ± 6.8 | 32.8 ± 5.7* | 169.8 ± 36.1* | 109.6 ± 25.3*† | 106.6 ± 25.6* | 7.3 ± 3.7* |
| Nonsquamous, regional (11) | 65.8 ± 6.1 | 29.6 ± 3.9 | 146.2 ± 25.2* | 87.0 ± 13.2*† | 90.5 ± 20.0* | 6.0 ± 1.4* |
| Cured clinically | | | | | | |
| Squamous (14) | 71.0 ± 7.5 | 30.5 ± 4.7 | 143.1 ± 19.5* | 85.8 ± 14.9 | 88.5 ± 18.4*† | 6.0 ± 2.0*† |
| Nonsquamous (29) | 68.7 ± 5.6 | 29.1 ± 3.7 | 131.9 ± 16.5* | 82.1 ± 9.8 | 76.4 ± 13.5*† | 4.7 ± 1.6*† |

* Significantly different from normal group, $p < .05$
† Significant difference between squamous and nonsquamous groups, $p < .05$

With tumor-bearing individuals classified as having either local, regional or systemic disease, the degree of elevation of the protein-bound carbohydrates became progressively greater as tumor burden increased (Table 1). The difference of each group from normal was significant. Further, the carbohydrate levels of the regional group were significantly elevated compared to the local group and the systemic group was elevated compared to the regional group, but

not significantly. The sialic acid and neutral hexose levels were significantly different in the group with localized tumors compared to the smoking controls.

In general, the mean carbohydrate levels in cured patients compared to normals were elevated (Table 1). When this group was divided into those cured of squamous and those cured of nonsquamous tumors, the elevations were primarily in the squamous group (Table 2). Compared to the smokers, only neutral hexose and sialic acid were significantly elevated in the group of patients cured of squamous tumors.

Correlation coefficients were determined for the carbohydrate moieties. The correlation between the individual serum protein-bound carbohydrates was highly significant when either the tumor-bearing group (Table 3) or all individuals in the study were considered.

| | Correlation coefficients | | |
|---|---|---|---|
| | Fucose | Sialic acid | Hexosamine |
| Neutral hexose | .845 | .943 | .915 |
| Hexosamine | .842 | .921 | |
| Sialic acid | .806 | | |

Table 3.
Correlation Between Glycoprotein Carbohydrate Moieties in Tumor-Bearers (62)

Both protein-bound carbohydrates and CEA were determined in the sera of some individuals. A comparison of the mean values of sialic acid, fucose, and CEA in patient groups and smokers, related to healthy normal values, is shown in Figure 1. The mean serum CEA value in ng/ml for the 27 non-smoking normals was $2.2 \pm 1.6$ (S. D.). The group of 15 heavy smokers had a mean CEA of $4.2 \pm 2.6$ (S. D.) and the levels were significantly different from normal ($p < .01$). In the tumor-bearers, the mean CEA values showed an increase with tumor burden except that the systemic group was lower than the regional group (Figure 1). The small group of patients with disseminated disease must play a part in this finding. The clinically cured patients had a mean CEA of $2.6 \pm 1.7$ (S. D.), similar to normal. With the exception of normals compared to heavy smokers, there were no statistical differences between the

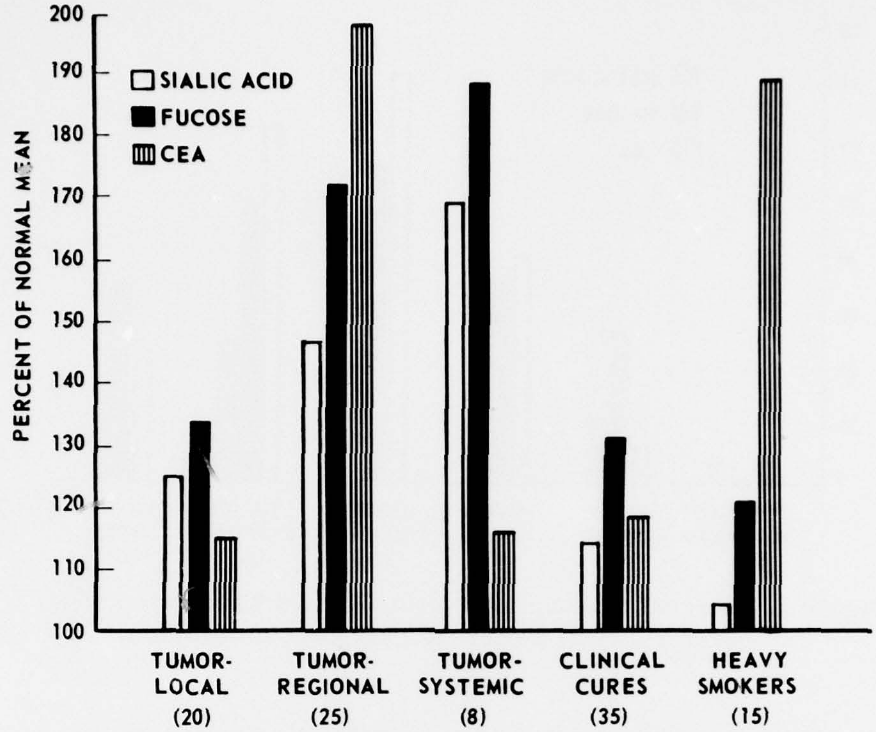


Figure 1. Mean values of tumor markers compared to normal

groups. It was convenient to define the upper limit of normal as 5.0 ng/ml, which is slightly less than the mean + two standard deviations for the 27 normal controls. The percentage of individuals in the population studied who had elevated CEA, sialic acid, or fucose (above normal mean + two standard deviations) is shown in Figure 2. There appeared to be little relation between elevated bound-carbohydrate and elevated CEA levels. While 65 percent of all tumor-bearers had either a CEA or sialic acid level above the normal mean + two standard deviations, only 9 percent of them had both markers elevated. Similarly, only 9 percent of the tumor-bearers had both CEA and fucose elevated, although there was a small but significant correlation between these two parameters in the group of 53 patients with tumors studied ($r = +.30$).

The mean values of the 18 specific normal serum proteins determined by radial immunodiffusion, divided into the tumor-bearing and cured groups, are

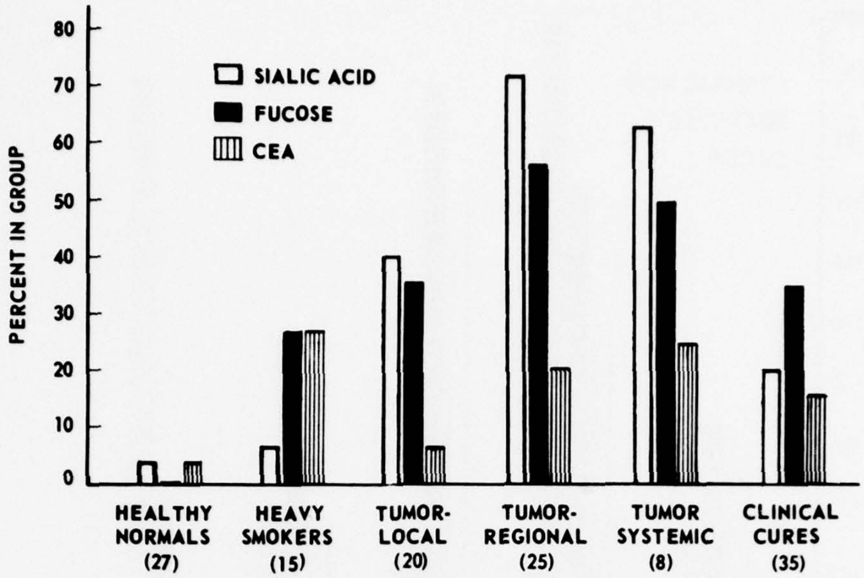


Figure 2. Percent of individuals with elevated tumor markers (above normal mean + two standard deviations).

presented in Table 4. When the 43 tumor-bearers were compared to controls, $\alpha_1$-antitrypsin, ceruloplasmin, haptoglobin, and hemopexin were significantly elevated, while albumin was significantly decreased. Comparison of tumor-bearing patients to the cured group evidenced further changes in serum proteins due to malignancy. Increases were found in $\alpha_1$-acid glycoprotein, $\beta_1$A-globulin, and $\beta_1$E-globulin; but a depression was evidenced in $\alpha_2$HS-glycoprotein. When the tumor-bearers were grouped as to extent of disease, there was a direct relationship between tumor burden and the increases in the acute-phase proteins ($\alpha_1$-acid glycoprotein, $\alpha_1$-antitrypsin, ceruloplasmin, and haptoglobin) (Table 5). When the tumor-bearers were grouped on the basis of histology into squamous and nonsquamous groups (Table 6), higher but not statistically significant levels of $\alpha_1$-acid glycoprotein, $\alpha_1$-antitrypsin, and haptoglobin were found in the group of patients with squamous tumors. However, the squamous tumor-bearers were found to have significant elevations of IgA ($p < .05$) and IgG ($p < .01$) compared to nonsquamous tumor-bearers. Transferrin levels appeared

Table 4. Serum Proteins in Patients With Solid Malignancies (Mean ± S. D.)

| | Normals (10) | Tumor-bearers (43) | Cures (39) |
|---|---|---|---|
| Albumin | 3950 ± 500 | 3470 ± 610*† | 3990 ± 475† |
| $\alpha_1$-acid glycoprotein | 75 ± 18 | 118 ± 72† | 77 ± 19† |
| $\alpha_1$-antitrypsin | 286 ± 82 | 386 ± 144*† | 282 ± 98† |
| Ceruloplasmin | 32 ± 6 | 40 ± 11*† | 34 ± 7† |
| Haptoglobin | 181 ± 95 | 321 ± 212*† | 206 ± 99† |
| Gc-globulin | 29 ± 5 | 30 ± 4 | 31 ± 5 |
| $\alpha_2$-macroglobulin | 301 ± 74 | 241 ± 99 | 254 ± 99 |
| $\alpha_2$ HS-glycoprotein | 58 ± 8 | 55 ± 16† | 63 ± 12† |
| $\beta$-lipoprotein | 599 ± 125 | 520 ± 107 | 549 ± 129 |
| Transferrin | 299 ± 46 | 255 ± 88 | 270 ± 70 |
| Hemopexin | 79 ± 13 | 93 ± 18*† | 82 ± 14† |
| $\beta_2$-glycoprotein | 22 ± 4 | 22 ± 4 | 21 ± 5 |
| $\beta_1$A-globulin | 86 ± 15 | 97 ± 22† | 87 ± 17† |
| $\beta_1$E-globulin | 38 ± 15 | 49 ± 19† | 38 ± 15† |
| $I_gM$ | 151 ± 72 | 169 ± 79 | 182 ± 87 |
| $I_gA$ | 227 ± 117 | 270 ± 136 | 255 ± 129 |
| $I_gG$ | 1316 ± 296 | 1277 ± 521 | 1322 ± 432 |
| Plasminogen | 14 ± 2 | 15 ± 3 | 14 ± 3 |

* Significantly different from normal group, $p < .05$
† Significant difference between tumor-bearers and cures, $p < .05$

to be lower in patients with nonsquamous tumor involvement, including the cured group (Table 6).

A correlation between serum-bound carbohydrates and individual serum proteins in tumor-bearers is given in Table 7. Similar levels of correlation were also observed when the total group studied (10 normals, 43 tumor-bearers, and 39 cures) was considered. The best positive correlations were with the acute-phase proteins while the best negative correlations were with $\alpha_2$HS-glycoprotein and albumin. For comparison, the significant correlation

Table 5. Serum Proteins in Patients With Solid Malignancies: Effect of Tumor Burden (Mean ± S. D.)

| | Normals (10) | Local (15) | Regional (19) | Disseminated (9) |
|---|---|---|---|---|
| Albumin | 3950 ± 500 | 3560 ± 610 | 3590 ± 550 | 3060 ± 600† |
| $\alpha_1$-acid glycoprotein | 75 ± 18 | 86 ± 40 | 116 ± 61* | 176 ± 101† |
| $\alpha_1$-antitrypsin | 286 ± 82 | 333 ± 73 | 373 ± 124 | 502 ± 209† |
| Ceruloplasmin | 32 ± 6 | 37 ± 8 | 40 ± 11* | 47 ± 14† |
| Haptoglobin | 181 ± 95 | 219 ± 97 | 345 ± 196* | 442 ± 310* |
| Gc-globulin | 29 ± 5 | 29 ± 4 | 31 ± 5 | 32 ± 5 |
| $\alpha_2$-macroglobulin | 301 ± 74 | 228 ± 46* | 258 ± 139 | 223 ± 56* |
| $\alpha_2$ HS-glycoprotein | 58 ± 8 | 58 ± 13 | 56 ± 13 | 49 ± 25 |
| $\beta$-lipoprotein | 559 ± 125 | 516 ± 118 | 506 ± 98 | 554 ± 109 |
| Transferrin | 299 ± 46 | 291 ± 95 | 261 ± 72 | 182 ± 72* |
| Hemopexin | 79 ± 13 | 88 ± 14 | 96 ± 22* | 95 ± 16* |
| $\beta_2$-glycoprotein | 22 ± 4 | 22 ± 5 | 23 ± 3 | 21 ± 5 |
| $\beta_1$A-globulin | 86 ± 15 | 91 ± 21 | 97 ± 22 | 108 ± 24* |
| $\beta_1$E-globulin | 38 ± 15 | 42 ± 11 | 47 ± 15 | 64 ± 29* |
| $I_gM$ | 151 ± 72 | 143 ± 56 | 184 ± 97 | 179 ± 69 |
| $I_gA$ | 227 ± 117 | 225 ± 103 | 314 ± 169 | 254 ± 69 |
| $I_gG$ | 1316 ± 296 | 1153 ± 453 | 1384 ± 524 | 1259 ± 626 |
| Plasminogen | 14 ± 2 | 15 ± 3 | 15 ± 3 | 15 ± 2 |

Significantly different from normal group:
* $p < .05$
† $p < .01$

coefficients obtained when protein-bound carbohydrates were compared to serum CEA are also given in Table 7.

Lymphocyte reactivity to PHA in both tumor-bearing and cured patients was determined and compared to lymphocyte reactivity levels in control individuals (Table 8). The mean lymphocyte reactivity was similar in healthy nonsmoking controls and in smoking normals, while the tumor-bearing patients' mean lymphocyte reactivity was significantly depressed compared to normal. When the patients were grouped as local or regional tumor-bearers, the difference from normal of each group was not significant. Most of the depression in lymphocyte reactivity in the tumor-bearing group was due to the patients with squamous cell carcinoma of the head and neck, and this group was significantly

Table 6. Serum Proteins in Patients With Solid Malignancies: Comparison of Patients With Squamous and Nonsquamous Tumors (Mean ± S. D.)

| | Squamous tumors (17) | Nonsquamous tumors (26) | Squamous cures (14) | Nonsquamous cures (25) |
|---|---|---|---|---|
| Albumin | 3540 ± 570 | 3430 ± 630* | 4060 ± 480 | 3950 ± 480 |
| $\alpha_1$-acid glycoprotein | 128 ± 83 | 111 ± 65 | 80 ± 20 | 76 ± 18 |
| $\alpha_1$-antitrypsin | 411 ± 137* | 370 ± 148 | 322 ± 125 | 260 ± 73 |
| Ceruloplasmin | 41 ± 11* | 40 ± 11* | 37 ± 7 | 33 ± 7 |
| Haptoglobin | 333 ± 232 | 314 ± 202 | 199 ± 111 | 210 ± 94 |
| Gc-globulin | 31 ± 5 | 30 ± 4 | 32 ± 6 | 30 ± 4 |
| $\alpha_2$-macroglobulin | 255 ± 145 | 231 ± 54* | 217 ± 91 | 275 ± 98 |
| $\alpha_2$ HS-glycoprotein | 55 ± 15 | 55 ± 17 | 67 ± 15 | 61 ± 10 |
| $\beta$-lipoprotein | 502 ± 120 | 531 ± 98 | 537 ± 96 | 556 ± 146 |
| Transferrin | 285 ± 109 | 235 ± 67* | 311 ± 87† | 247 ± 47† |
| Hemopexin | 94 ± 23 | 92 ± 15* | 87 ± 13 | 79 ± 13 |
| $\beta_2$-glycoprotein | 24 ± 4 | 21 ± 5 | 22 ± 7 | 21 ± 5 |
| $\beta_1$A-globulin | 93 ± 16 | 100 ± 25 | 85 ± 19 | 89 ± 16 |
| $\beta_1$E-globulin | 52 ± 14* | 47 ± 22 | 43 ± 19 | 35 ± 11 |
| $I_gM$ | 170 ± 74 | 168 ± 84 | 182 ± 109 | 182 ± 75 |
| $I_gA$ | 343 ± 158† | 223 ± 95† | 294 ± 135 | 234 ± 122 |
| $I_gG$ | 1482 ± 572† | 1144 ± 447† | 1387 ± 434 | 1285 ± 436 |
| Plasminogen | 14 ± 3 | 15 ± 3 | 15 ± 3 | 14 ± 3 |

* Significantly different from normal group, $p < .05$

† Significant difference between squamous and nonsquamous groups, $p < .05$

different from normal. The mean lymphocyte reactivities showed a decrease with increasing tumor burden and a return to normal in the cured patient. This pattern suggests an inverse relationship with protein-bound carbohydrates. When compared by regression analysis, the inverse correlations were not statistically significant. However, lymphocyte reactivity was significantly correlated with some serum proteins measured by radial immunodiffusion. The most significant correlation was with $\alpha_2$HS-glycoprotein (positive) and to a lesser extent with albumin (positive) and some of the acute-phase proteins (negative) (Table 9).

Table 7. Correlation Between Protein-Bound Carbohydrates and Specific Serum Proteins in Tumor-Bearers (43)

| | Correlation coefficients | | | |
|---|---|---|---|---|
| | Neutral hexose | Hexosamine | Sialic acid | Fucose |
| Albumin | -.54 | -.58 | -.63 | -.39 |
| $\alpha_1$-acid glycoprotein | .93 | .89 | .95 | .86 |
| $\alpha_1$-antitrypsin | .88 | .91 | .90 | .75 |
| Ceruloplasmin | .70 | .68 | .74 | .62 |
| Haptoglobin | .89 | .86 | .86 | .76 |
| Gc-globulin | .42 | .31 | .39 | NS |
| $\alpha_2$-macroglobulin | NS | NS | NS | NS |
| $\alpha_2$ HS-glycoprotein | -.57 | -.62 | -.64 | -.51 |
| $\beta$-lipoprotein | NS | NS | NS | NS |
| Transferrin | -.38 | -.44 | -.46 | -.36 |
| Hemopexin | .55 | .52 | .55 | .49 |
| $\beta_2$-glycoprotein | NS | NS | NS | NS |
| $\beta_1$A-globulin | .40 | .30 | .34 | NS |
| $\beta_1$E-globulin | .49 | .45 | .52 | .30 |
| $I_gM$ | .39 | .40 | .32 | .41 |
| $I_gA$ | .44 | .47 | .39 | .38 |
| $I_gG$ | .53 | .46 | .43 | .59 |
| Plasminogen | NS | NS | NS | NS |
| CEA | .28 | NS | NS | .30 |

NS = correlation not significant ($p < .05$)

## DISCUSSION

Elevations in one or more of the glycoprotein-associated carbohydrates in the sera of cancer patients have been reported by a number of investigators.[1,5,14,15,23,27,29,32,43,45]

Only a few studies have compared increases in each of the protein-bound carbohydrates, and values for patients with varying degrees of tumor extension have not always been given. In the present study, each of the protein-bound carbohydrates (neutral hexoses, hexosamines, sialic acid, and fucose) has

Table 8. Lymphocyte Reactivity in Patients With Solid Tumors

| | Lymphocyte reactivity* |
|---|---|
| Healthy normals (27) | 80.9 ± 25.5 |
| Heavy smokers (15) | 79.3 ± 17.5 |
| Tumor-bearing | |
| Local (15) | 69.2 ± 24.2 |
| Regional (20) | 66.3 ± 25.6 |
| Squamous (24) | 63.5 ± 24.1† |
| Nonsquamous (11) | 76.3 ± 24.8 |
| All tumor-bearers (35) | 67.5 ± 24.7† |
| Cured clinically | |
| Squamous (14) | 74.4 ± 20.1 |
| Nonsquamous (9) | 89.6 ± 26.9 |
| All cures (23) | 80.3 ± 23.7 |

* Mean incorporation ± standard deviation of tritiated thymidine in dis/min x $10^{-3}$ of lymphocytes cultured with PHA

† Significantly different compared to healthy normals, $p < .05$

Table 9. Correlation of Lymphocyte Reactivity With Serum Proteins

| | Correlation coefficients | | |
|---|---|---|---|
| | Tumor-bearers, cured and normal (53) | Tumor-bearers and cured (46) | Tumor-bearers (23) |
| $\alpha_1$-antitrypsin | -.24 | -.30* | -.29 |
| Haptoglobin | -.29* | -.28 | -.23 |
| $\alpha_1$-acid glycoprotein | -.22 | -.23 | -.15 |
| $\alpha_2$-macroglobulin | +.26 | +.25 | +.37 |
| Albumin | +.32* | ±.29 | +.34 |
| $\alpha_2$ HS-glycoprotein | +.37† | +.47† | +.60† |

Significant correlation:
* $p < .05$
† $p < .01$
Twelve other proteins and CEA showed no significant correlation with lymphocyte reactivity

been shown to be related to tumor burden in a group of patients with solid malignancies. Further, significant differences in bound carbohydrates were found between controls and those patients with localized tumors. Serum glycoprotein carbohydrates also appear to be affected by tumor site or histology (squamous versus nonsquamous). From the data, it is apparent that the levels of each carbohydrate measured are related and reflect tumor burden similarly; each increases as tumor burden increases. Each of the carbohydrate moieties correlates well with the others (Table 3), although fucose concentrations appear to be more independent, as reflected by the slightly lower correlation of this carbohydrate with the others.

In patients clinically free of disease two years following therapy, the mean carbohydrate levels, except for hexosamine, were significantly elevated compared to healthy normals (Tables 1 and 2). Attempts to distinguish patients free of disease from those with localized disease by means of protein-bound carbohydrate levels would prove difficult (Tables 1 and 3). If the carbohydrate levels in each patient in the cured group are elevated (Figure 2), some patients' levels are entirely normal, while other patients have elevated levels despite apparent cure of their tumors. Elevations in the carbohydrate levels in cured patients are greater in patients with squamous cell carcinoma, a similar case as in tumor-bearing patients (Table 2). Tumor site as well as histology may also be a contributing factor to the higher levels of carbohydrates in patients with squamous tumor involvement. The known environmental factors associated with squamous carcinoma of the head and neck region (alcohol consumption and cigarette smoking[30]), may play a role in the observed elevated carbohydrate levels. Heavy smoking is associated with modest elevations in some of the glycoprotein-associated carbohydrates (Table 1). This might be explained on the basis of the reaction to the subclinical bronchiolitis caused by smoking.

In the present study, we have addressed the question of the source of the increased protein-bound carbohydrates occurring in cancer. The major

possibilities that must be considered are (1) increased synthesis of glycoprotein by the liver and/or lymphoreticular tissue, (2) increased carbohydration of normal proteins, or (3) glycoprotein production by the tumor itself. In regard to the first possibility, we have measured the levels of 17 normal serum glycoproteins and compared them to the levels of protein-bound carbohydrates. The present study and the work of Snyder and Ashwell[38] provide data on changes in most of the normal serum proteins during neoplasia. The elevations in $\alpha_1$-antitrypsin, haptoglobin, $\alpha_1$-acid glycoprotein, ceruloplasmin, and hemopexin, found by Snyder and Ashwell[38] in patients with various types of cancer, were corroborated in our study of a larger group of patients with solid malignancies. Comparison of tumor-bearers and cured patients also points out the possible importance of $\beta_1$A- and $\beta_1$E-globulin, $\alpha_2$HS-glycoprotein, and transferrin levels in cancer patients. In addition, higher levels of IgG and IgA were noted in the sera of patients with squamous tumors compared to patients with nonsquamous tumors. It has been suggested that increased IgG or IgA concentrations occur in those cancer patients where the malignant tissues involved are continually exposed to bacterial invasion.[18]

In the present study, the acute-phase proteins ($\alpha_1$-acid glycoprotein, $\alpha_1$-antitrypsin, ceruloplasmin, and haptoglobin) were shown to be related to tumor burden in a manner similar to the protein-bound carbohydrates. Furthermore, there was excellent correlation (Table 7) between the measured carbohydrates and these acute-phase proteins, which are known to contain very large amounts of carbohydrate.[35] From these considerations, we conclude that the majority of the increased protein-bound carbohydrates that we have measured come from liver-produced normal serum proteins, especially the acute-phase proteins of the $\alpha$-globulin fraction of the serum. Levels of acute-phase proteins rise within 12-36 hours after and in response to tissue injury, infection, trauma, and inflammation.[21] The mechanism causing the acute-phase response is not known. In neoplasia, it is possible that tissue destruction is the stimulus for the acute-phase reaction.

Although fucose levels correlate well with the levels of acute-phase proteins, the correlation is not as good as the correlation of sialic acid and the acute-phase proteins (Table 7). Conversely, since the ratio of fucose to sialic acid is greater in the immunoglobulins than in the $\alpha$-globulins,[35] fucose levels are also correlated with immunoglobulin levels. Therefore, fucose may reflect both immunoglobulin levels and acute-phase proteins, while sialic acid reflects mainly the latter.

Some investigators have suggested that increased levels of chemically-measured carbohydrate in the serum of rats with tumors is elaborated by the tumor.[20,43] Studies using labeled glycoprotein precursors indicate that although the liver is the major source of synthesized serum protein, transplantable tumors in hepatectomized rats can also synthesize serum glycoproteins.[6,24] There is no evidence, at the present time, that glycoproteins of tumor origin make a significant quantitative contribution to the increased levels of protein-bound carbohydrate or $\alpha$-globulins found in humans with cancer. Tumor-associated glycoproteins, such as CEA, HCG, and $\alpha$-fetoprotein, would not be expected to contribute significantly to serum-bound carbohydrates since they are measured in nanogram quantities and total serum protein-bound carbohydrates occur in milligram quantities. However, the possibility that tumors secrete proteins that are normally found in the sera cannot be completely excluded. Other sources of serum carbohydrate (glycolipids and acid mucopolysaccharides) that may increase in neoplasia also occur in very low concentrations compared to normal serum glycoproteins.

The final possibility of increased carbohydration of normal proteins in cancer patients does not have strong support from past studies. The sialic acid content of an $\alpha_1$-acid glycoprotein fraction isolated from the sera of cancer patients was found in some cases to be lower than normal.[31,34,48] Alterations in liver glycoprotein synthesis might occur in malnutrition,[25] and this would be a consideration in some patients with advanced cancer.

In determining the success of a tumor marker in accurately indicating tumor burden, it is of value to compare it with another marker. A comparison between bound carbohydrates and serum CEA as indicators of tumor burden was made. Although increases in either CEA or total-bound carbohydrate are not always due to the presence of a tumor, CEA was considered to be representative of tumor-specific or tumor-derived markers, while bound carbohydrates were considered to be representative of a nonspecific response to neoplasia. Sialic acid appears to be a better tumor marker than CEA or fucose in the group of patients with various types of tumors studied by us. Sialic acid has the highest positive rate in the tumor-bearers, a low incidence of false positives in smokers, and a comparable number of false positives in the cured group (Figure 2). There is a small correlation between CEA levels and bound carbohydrate levels; however, elevated CEA levels do not correlate well with elevated carbohydrate levels. Similarly, Crawley et al.[13] compared CEA levels with levels of seromucoid, some acute phase proteins, and immunoglobulins. They could not establish a direct relationship between the concentration of CEA and the other parameters. Since there is so little correlation between increased CEA and bound carbohydrate levels, the use of both nonspecific protein-bound carbohydrate levels and assays of CEA or other tumor-specific antigens may provide a better insight into patient status.

Previous work has indicated that changes in $\alpha$-globulins during neoplasia may have immunological significance.[12,19] Watkins et al.[43] reported a strong correlation between serum-bound sialic acid of cancer sera and lymphocyte transformation response to neuraminidase-treated tumor cells _in vitro_; however, a similar correlation did not hold true for mitogen and alloantigen-induced lymphocyte transformation using sera from patients previously shown to be suppressive.[15] Apffel and Peters[3] have postulated that the increased levels of liver-produced sialoglycoproteins are nonspecifically immunosuppressive. The present study cannot give direct support to these views, but considers briefly the relationship between the serum parameters studied by us and one parameter

of immunological response. The lowered lymphocyte reactivity to PHA in cancer patients, especially those with squamous tumors shown in the present study, agrees with previous results from this laboratory that indicate that patients with squamous tumors have a greater impairment of immune competency as measured by a variety of immunological techniques.[9,33,41] The present study shows, in general, that groups with lowered lymphocyte reactivity to PHA have increased mean sialic acid levels (Tables 1, 2, and 3). However, we found no significant inverse correlation between levels of protein-bound carbohydrates and lymphocyte reactivity. Significant negative correlations between lymphocyte reactivity and specific acute-phase proteins were found (Table 9). Further, a positive correlation was found between lymphocyte reactivity and $\alpha_2$HS-glycoprotein, a protein whose function is unknown. These results suggest that further studies on the immunological aspects of changes in specific serum glycoproteins in neoplasia are needed, including studies on the relative clinical utility of the biochemical markers and immunological assays as indicators of tumor burden.

The search for a biochemical test to detect the presence or extent of a tumor has led to the development of many assays that appear to us to be related to the increased liver synthesis of acute-phase proteins. These include analyses of seromucoid[17] (mainly $\alpha_1$-acid glycoprotein), a colorimetric test using Ehrlich's reagent[28] (probably measuring sialic acid), immunological[38] or functional[11] measurements of individual globulins, and the analysis of the individual protein-bound carbohydrates. Recent procedures that will probably fall into this category are the measurement of serum levels of carbocyanine dye-binding polyanions[47] and of glycosyltransferase activities.[8,26,44] The definite changes in metabolism of glycoproteins and the altered levels of related enzymes in tumor cells[42] have encouraged the search for these parameters in the blood. However, we would not expect to find these changes readily by biochemical procedures due to the overwhelming contribution of the liver to serum glycoprotein metabolism, which is also greatly altered in neoplasia.

Measurements of protein-bound carbohydrates such as sialic acid (or other nonspecific indicators of the acute-phase response) may have clinical utility, despite their limitations, and especially if used in conjunction with measurements of other tumor markers. Protein-bound carbohydrates may be useful as indicators of tumor burden prior to therapy in patients with solid malignancies. Carbohydrate levels could be used to verify clinical staging to identify those patients who should be considered as high risk and incorporated into an adjuvant therapy group, or to alert the clinician to the possibility of unrecognized occult disease.

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